



Characterization of bacteria associated with untreated otitis media with effusion among primary school children in Kano Metropolis, Nigeria

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Otitis media with effusion is an inflammation of the middle ear in which fluid accumulates behind the eardrum. The aim of the study was to determine the bacteria isolates associated with untreated otitis media with effusion among primary school children in Kano metropolis Nigeria. A total of 42 samples were collected using sterile swab sticks from the subjects with symptoms of otitis media with effusion among primary school children within Kano metropolis from April 2017 – July 2017. The samples were inoculated onto the surface of Nutrient agar, Mannitol salt agar, Chocolate agar and MacConkey agar. Identification of the isolates was done using Gram staining, bacteriological analysis and Biochemical tests. A total of 189 (8 species) isolates were recovered from 42 samples. The isolates were *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *H. influenza* and *Staphylococcus epidermidis*. *Staphylococcus aureus* has the highest prevalence with 34 isolates which accounted for 18%, this is followed by *E. coli* with 26 isolates and accounted for 15% while least number of isolates (17) was recorded in *Staphylococcus epidermidis* which accounted for 9%. It is concluded that Otitis media is polymicrobial infection.

INTRODUCTION

The ear is a sense organ concerned with hearing and balance. Sound waves transmitted from outside enters the ear. It is made up of three parts, the outer, middle and inner ear [1]. Otitis media is the inflammation of middle ear cavity. Otitis media can be caused by infections, allergy, anatomic or functional deviations of the middle ear or Eustachian tube [2]. The ear infection can be acute (acute otitis media) or chronic (chronic or suppurative otitis media). Acute otitis media is a middle ear effusion (fluid) associated with symptoms of pain, fever and irritability. Otitis media with effusion, often referred to as glue ear, describes fluid in the middle ear with no sign of fever or inflammation of the ear drum [3]. Children are mostly infected with otitis media since the Eustachian tube is short and at more of a horizontal angle than in the adult ear [4]. They also have not developed the same resistance to bacteria, viruses and fungi as adults. The Eustachian tube is usually closed but opens regularly to ventilate or replenish the air in the middle ear. This tube also equalizes middle ear air pressure in response to air pressure changes in the environment. However, Eustachian tube that is blocked by swelling of the lining or

plugged with mucous from a cold or from other reason cannot open to ventilate the middle ear [5].

Infections usually results from bacterial and fungal causes, and in some cases secondary to other viral infections like upper respiratory tract infections (URTI) [6]. It may result in serious complications as mastoiditis, meningitis or intracranial abscess [7]. In the pre-antibiotic era of the early 1900s, b-haemolytic group A *Streptococcus pyogenes* was the most common frequently isolated pathogen from people with acute otitis media (AOM), especially when AOM was a complication of acute tonsillitis or scarlet fever [8,9]. However, since the 1950s, it has been rapidly replaced by *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* [8,10] which are nowadays the main bacterial pathogens followed by other less frequent pathogens as *S. aureus*, and more rarely, anaerobic Gram negative bacilli such as *Escherichia coli* or *Pseudomonas aeruginosa* [11,12]. According to Glezen [13], the most common bacteria causes for acute otitis media are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The incidence of penicillin resistant *Streptococcus pneumoniae* has risen, but rates vary between countries. Others are *Escherichia coli*, *Staphylococcus epidermidis* etc. while the fungal likely agents are; *Aspergillus niger* and *Candida* species. Viruses such as respiratory syncytial virus (RSV) and those that cause common cold may also result in otitis media by damaging the normal defenses of the epithelial cells in the upper respiratory tract.

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With the advent of antibiotics, the incidence of complications had decreased. However, due to unnecessary and improper use of antibiotics, resistance by bacteria, and increase in susceptibility of individuals, complications still occur. The acquired knowledge about the prevalence of microorganisms responsible for or involved in otitis media with effusion cases may help select the most appropriate antimicrobials and minimize complications. The aim of the present study was to determine the prevalence of bacteria associated with untreated otitis media with effusion among primary school children in Kano metropolis, Nigeria.

MATERIALS AND METHODS

Study Area

Kano State is a state located in North-Western Nigeria and one of the largest State of the Nigerian Federation, Created on May 27, 1967 from part of the Northern Region. Kano state is bordered by Katsina state to the North-West, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west. Kano is located on 12° N and 8°30'E. It has a total area of 20,131 km². The urban area covers 137km² and comprises of six LGAs - Kano municipal, Fagge, Dala, Gwale, Tarauni and Nassarawa with population of 2,163,25 as at 2006 [14]. Climate of the study areas have been described as 'AW' type as identified by Koppen's climatic classification [15]. The vegetation is a Savanna type simply described as closed grass or other predominantly herbaceous vegetation with scattered or widely spaced woody plants. Vegetation types in the state are the northern Guinea savanna and Sudan savanna. Northern Guinea Savanna is open woodland with grasses shorter than in the southern guinea where grasses are 1.5 to 3m tall. The Sudan Savanna has scattered trees in open grassland with grasses under 1.2m tall. The vegetation has been largely cleared for cultivation to form cultivated parkland. Parkland has scattered protected trees at some distance apart in open cultivated land [15].

Collection of Samples

The study population consisted of 42 primary school children aged between 7-12 years with untreated middle ear effusion. A total of 42 samples were collected from the subjects with symptoms of otitis media with effusion among primary school children within Kano metropolis from April 2017 – July 2017. During collection, the external auditory canals of the subjects were sterilized with 70% alcohol. Samples were collected using sterile swab stick and transported to Microbiology Laboratory of Bayero University Kano for bacteriological analysis.

Isolation of bacteria isolates

The clinical wound swab samples were inoculated onto Nutrient agar (Life save Biotech, USA), Mannitol salt agar (Biomark, India), Chocolate agar and Mac Conkey agar (Life save Biotech, USA) plates and incubated aerobically at 37⁰ C for 24 hours. After incubation bacterial growth was observed for colony appearance and morphology. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained.

Identification of Bacterial Isolates

Presumptive colonies were confirmed by gram staining, biochemical (Indole, Methyl-red, Vogues Proskeaur, Citrate utilization Catalase and Oxidase) tests, nitrate reduction, sugar fermentation and motility test. Each plate was graded as positive or negative. Bacteria isolates were characterized according to Bergy's manual of systemic determinative Bacteriology by Holt *et al.* [16].

RESULTS

Identification of Bacterial Isolates

The identification of the bacteria isolates using Gram staining, biochemical characterization and sugar fermentation is presented in table 2. The result indicated that, a total of 8 isolates were identified namely; *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *H. influenza* and *Staphylococcus epidermidis*.

Prevalence of Bacterial isolate

The prevalence of the bacteria isolate from untreated otitis media with effusion from 42 subjects is presented in table in table 3. A total of 189 isolates were recovered. *Staphylococcus aureus* has the highest prevalence with 34 isolates which accounted for 18%, this is followed by *E. coli* with 26 isolates and accounted for 15% while least number of isolates (17) was recorded in *Staphylococcus epidermidis* which accounted for 9%.

DISCUSSION

The study was aimed to identify some bacteria associated with untreated otitis media with effusion among primary school children in Kano metropolis, Nigeria. The analysis of study population used in this research showed that more male subjects were infected with otitis media with effusion than female subjects, as 23 males was recorded against 19 females. The mechanisms involved for such sexual dimorphisms are multi-factorial, including the endocrine and genetic effects on the immune system and physiology, as well as sex-related differences in behavior. In humans, females reportedly mount stronger humoral and cellular immune responses to infection or antigenic stimulation than do males [17]. This result was contrary to that of Jik *et al.* [18] who found that females (with total percentage isolates of 54.16%) were more susceptible to infection of the middle ear than the males (45.84%) although statistically, there was no significant difference ($p>0.05$). The result also disagree with that of Olubanjo [19] who conducted research in Kupa medical centre with prevalence rates of 47.1% for males and 52.9% for females. The age distribution of the subjects (Table 1) revealed that otitis media with effusion is more frequent among children with less number of age. There are many reasons why children are more likely to suffer from otitis media than adults. Children have more trouble fighting infections; this is because their immune systems are still developing [20]. Infants in whom otitis media with effusion develop in the first year of life have an increase risk of recurrent middle ear infection. According to this study, the overall prevalence of the disease tends to decrease with age especially after the age of 7. There are several factors responsible for transmission of otitis media among children, such factors include; overcrowding, poor nutrition, poor hygiene, and lack of attention to symptoms may also increase the incidence, type and severity of otitis media.

In the present study, several methods including Gram staining, biochemical characterization and bacteriological analysis using selective and differential agar medium were deployed for characterization of bacterial isolates. A total of 189 bacteria isolates were recovered from 42 different samples of effusions from untreated otitis media infection. Out of 189 bacterial pathogens isolated from 42 samples, *Staphylococcus aureus* was the most frequently isolated organism with total of 34 isolates which accounted for 18%. The prevalence rates of other isolates in according to this study were *E. coli* 15% (28 isolates), *Klebsiella pneumoniae* 14% (26 isolates), *Streptococcus pneumoniae* 12% while least percentage prevalence were recorded in *S. epidermidis*

Table 1 Age and sex distribution of the subjects

Age (years)	Male	Female	Total	Percentage (%)
7 – 8	6	5	11	26
8 - 9	5	5	10	24
9 - 10	4	4	8	19
10 - 11	5	3	8	19
11 - 15	3	2	5	12
Total	23	19	42	100

Table 2 Identification of Bacterial Isolates from untreated otitis media with effusion

Tests	A	B	C	D	E	F	G	H
GS	-	+	-	+	-	-	-	+
SH	Rod	Coccus	Rod	Coccus	Rod	Coccus	Spiral	Rod
IN	+	+	-	-	-	-	+	-
MR	+	+	-	+	-	+	-	-
VP	-	-	+	+	-	-	+	+
CU	-	-	+	+	+	+	+	-
CA	+	-	+	+	+	+	+	+
CO	-	-	-	+	-	-	-	-
OX	-	-	-	-	+	-	+	-
MO	+	-	-	-	+	+	+	-
NT	+	+	+	+	+	+	+	+
MF	+	-	+	+	+	-	-	-
LF	+	+	+	+	-	-	-	+
Organism	<i>E. coli</i>	<i>S. pneum</i>	<i>Kleb pne</i>	<i>S. aureus</i>	<i>P. aerugi</i>	<i>P. mirab</i>	<i>H. influe</i>	<i>S. epider</i>

Key: GS=Gram staining, SH= Shape, IN =Indole, MR=Methyl-red, VP=Vougues Proskeaur, CU=Citrate Utilization, CA=Catalase, CO=Coagulase, OX=Oxidase, MO=Motility, Nitrate reduction test, MF=Mannitol fermentation, LF=Lactose fermentation

Table 3 Prevalence of Bacterial isolate from untreated otitis media with effusion

Bacteria isolate	No. of isolates	Percentage prevalence (%)
<i>Staph aureus</i>	34	18
<i>E. coli</i>	28	15
<i>Klebsiella pneumoneae</i>	26	14
<i>Streptococcus pneumoneae</i>	23	12
<i>Pseudomonas aeruginosa</i>	21	11
<i>Proteus mirabilis</i>	21	11
<i>Haemophilus influenza</i>	19	10
<i>Staphylococcus epidermidis</i>	17	09
Total	189	100

9% with total of 17 isolates. The result of this study was in conformity with that of Jik et al. [18] who found *Staphylococcus aureus* as the most frequent isolates among children with otitis media infection in Ganawuri Area of Plateau State Nigeria. The predominance of *Staphylococcus aureus* in this study is similar to that found by Iseh and Adegbite [21] who have found that *S. aureus* (46.2 per cent) was the commonest organism cultured in their study [14]. On the other hand, this study was contrary to that of Gibney et al. [22] who found *Streptococcus pneumoniae* was the most cultured organism (82 per cent). In another study conducted in Soa Paulo, Brazil, *Streptococcus pneumoniae* accounted for 16 per cent of the pathogens isolated from Brazilian children with acute otitis media, whereas *Staphylococcus aureus* accounted for only 1 percent. This result is also contrary to the present study. The difference in the prevalence of bacteria isolates may be due to the geographical variability [21].

CONCLUSION

Otitis media is the inflammation of middle ear cavity and children are mostly infected the infection. According to this study, more male subjects were infected with otitis media with effusion than female subjects. Characterization of bacterial isolates from effusion of otitis media infection among children without treatment revealed the presence of *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoneae*,

Streptococcus pneumoneae, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *H. influenza* and *Staphylococcus epidermidis*. *Staphylococcus aureus* was the most frequently isolated organism according to this study. It is concluded that Otitis media is polymicrobial infection.

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